Biomarkers of Human Exposure to Pesticides

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For centuries, several hundred pesticides have been used to control insects. These pesticides differ greatly in their mode of action, uptake by the body, metabolism, elimination from the body, and toxicity to humans. Potential exposure from the environment can be estimated by environmental monitoring. Actual exposure (uptake) is measured by the biological monitoring of human tissues and body fluids. Biomarkers are used to detect the effects of pesticides before adverse clinical health effects occur. Pesticides and their metabolites are measured in biological samples, serum, fat, urine, blood, or breast milk by the usual analytical techniques. Biochemical responses to environmental chemicals provide a measure of toxic effect. A widely used biochemical biomarker, cholinesterase depression, measures exposure to organophosphorus insecticides. Techniques that measure DNA damage (e.g., detection of DNA adducts) provide a powerful tool in measuring environmental effects. Adducts to hemoglobin have been detected with several pesticides. Determination of chromosomal aberration rates in cultured lymphocytes is an established method of monitoring populations occupationally or environmentally exposed to known or suspected mutagenic–carcinogenic agents. There are several studies on the cytogenetic effects of work with pesticide formulations. The majority of these studies report increases in the frequency of chromosomal aberrations and/or sister chromatid exchanges among the exposed workers. Biomarkers will have a major impact on the study of environmental risk factors. The basic aim of scientists exploring these issues is to determine the nature and consequences of genetic change or variation, with the ultimate purpose of predicting or preventing disease. — *Environ Health Perspect* 105(Suppl 4):801–806 (1997)

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Introduction

Pesticides are ubiquitous contaminants of our environment and have been found in air, soil, water, and human and animal tissues in samples from all over the world. They cover a wide range of compounds used in pest control, including insecticides (arthropods), fungicides (fungi), herbicides (weeds), rodenticides (rats), molluscicides (snails), and others. A pesticide is defined as any substance or mixture of substances intended for preventing, destroying, or controlling any pest, including vectors of human or animal diseases, unwanted species of plants or animals that cause harm during the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products, or animal feedstuffs, or which may be administered to animals for the control of insects, arachnids, or other pests in or on their bodies (*1*).

Insecticides comprise a higher proportion of the total pesticide usage in developing countries than in developed countries. The principal classes of compounds that have been used as insecticides are organochlorine, organophosphorus, carbamate and pyrethroid compounds, and various inorganic compounds.

Pesticide uptake occurs mainly through the skin and eyes, by inhalation, or by ingestion. The fat-soluble pesticides and, to some extent, the water-soluble pesticides are absorbed through intact skin. Sores and abrasions may facilitate uptake through the skin. The vapors of pesticides or aerosol droplets smaller than 5 μm in diameter are absorbed effectively through the lungs. Larger inhaled particles or droplets may be swallowed after being cleared from the airways. Ingestion can also occur from the consumption of contaminated food or from using contaminated utensils. Contaminated hands may also lead to an intake of pesticides, for example, from cigarettes (*2*). Occupational exposures occur in the mixing and loading of equipment and in the spraying and application of insecticides. Absorption resulting from dermal exposure is the most important route of uptake for exposed workers. Acute toxic effects are easily recognized, whereas the effects resulting from long-term exposure to low doses are often difficult to distinguish. In particular, the effects of a regular intake of pesticide residues in food are hard to detect and quantify (*2*).

The improper use of pesticides may engender biological effects beyond those for which they were originally manufactured. Adverse effects may be caused not only by the active ingredients and the associated impurities, but also by solvents, carriers, emulsifiers, and other constituents of the formulated product (*2,3*).

Humans are diverse in their responses to exogenous exposures because of variability in the rate of metabolism, DNA repair processes, and other factors. The balance between activating and inactivating enzyme systems govern the rate of delivery of reactive metabolite to the target site (*4*). Several studies showed an association between health effects and exposure to insecticides (*5*). Disorders of the cardiovascular system, nervous system, sensory organs, and respiratory system, and reduced lung function have been reported after exposure to pesticides. Skin disorders, including dermatitis, headache, and nausea, have also been reported. Abnormal electroencephalograms were observed in some studies of farm workers exposed to organochlorine, organophosphorus, and carbamate insecticides. Altered liver enzyme activities have been reported among pesticide workers exposed to organophosphorus pesticides alone or in combination with organochlorine or other pesticides (*5*).

Transplacental transfer of carcinogens may induce damage to fetal tissue (*6*).

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Abbreviations used: AChE, acetylcholinesterase; δ-ALAD, δ-aminolevulinic acid dehydratase; AP, acephate; CA, chromosomal aberration; Cd, cadmium; ChE, cholinesterase; DGA, D-glucaric acid; MH-30, maleic hydrazide; PCD, polychlorinated dioxin; PCDF, polychlorinated dibenzofuran; RBC, red blood cell; SCE, sister chromatid exchange; SOD, superoxide dismutase.

Excessive spontaneous abortions occurred among couples exposed to several pesticides in grape gardens in India (*7*). Increased risks for spontaneous abortion and decreased birth weight were reported (*8*) in a population in Colombia where exposure to many pesticides occurred.

Genotoxic effects are considered among the most serious of the possible side effects of agricultural chemicals. If a chemical reacts to nuclear DNA, it is usually mutagenic and carcinogenic to the exposed organisms. The effects include heritable genetic diseases, carcinogenesis, reproductive dysfunction, and birth defects.

Several epidemiologic studies were performed on cancer risk after exposure to insecticides in countries all over the world, (*5*). The risk for multiple myeloma was greater for farmers residing in countries where insecticides were more heavily used (*9*). A cohort of workers from a large pest control company in the United States had an excessive lung cancer risk (*10*). Similarly, in a cohort of licensed pest control workers from Florida, there was significantly increased mortality from lung cancer (*11*). A follow-up of deaths among plant protection workers and agronomists in eastern Germany showed an increased risk of lung cancer, which also increased with the length of exposure (*12*). Among farmers licensed for pesticide use in the Piedmont region of Italy, increased risks for skin cancer and malignant lymphomas were reported (*13*). A cohort of pesticide appliers in Sweden showed excess risks for cancers of the lip and testis (*14*). In a study of a large cohort of grain millers in the United States, flour-mill workers had higher risks for non-Hodgkin's lymphoma and pancreatic cancer (*15*). The risk of non-Hodgkin's lymphoma increased with frequency of use of organophosphorus insecticides among farmers in Nebraska (*16*). In a study in Washington state, non-Hodgkin's lymphoma was associated with potential contact with chlordane and DDT (*17*). DDT use was also associated with non-Hodgkin's lymphoma in one of two studies in Sweden (*18*).

Biomarkers

The term biomarker is used to include almost any measurement reflecting an interaction between a biological system and an environmental agent, which may be chemical, physical, or biological. Biomarkers can be used to identify causal associations and to make better quantitative estimates of those associations at relevant levels of exposure (*19*). They may also make it possible to identify susceptible groups or individuals who are at risk of exposure to certain types of environmental and occupational agents (*4*).

Biomarkers include detection of the environmental substance itself or its metabolites in urine or blood, changes in genetic material, and cell death. The biological events detected can represent variation in the number, structure, or function of cellular or biochemical components. Recent advances in molecular and cellular biology allow for measurement of biologic events or substances that may provide markers of exposure, effect, or susceptibility in humans. Certain tests, such as DNA adduct formation, are used for measuring biologically effective dose, whereas others are considered to measure early effects, such as chromosomal aberrations (*20*). The ability to sequence DNA will revolutionize mutation research and lead to insights into the relationship between exposure and disease development. Genetic toxicology data could be coupled with medical information to diagnose disease onset and to develop therapeutic strategies (*21*).

Pesticides and their metabolites can be measured in biological samples, serum, fat, urine, blood, or breast milk by the usual analytical techniques or by biological methods (Table 1). A number of reports are available in which insecticides and/or their metabolites have been measured in body fluids after occupational exposures (*22–24*). Examples include the measurement of dialkylphosphates in urine after exposure to organophosphorus insecticides (*22*), of *p*-nitrophenol after exposure to parathion and methylparathion (*25*), and of 1-naphthol after exposure to carbaryl (26).

Studies have shown that numerous persistent organochlorine pesticides can be found in human fat. The reported levels vary among countries, the highest levels of DDT being found in countries where the compound is still used (*27*).

Many investigations of human milk contamination have been conducted worldwide. DDT and other organochlorine pesticides have been detected in most of the investigations. The contaminants found most frequently in human milk have been DDT, its main metabolite DDE, hexachlorobenzene, hexachlorocyclohexane, dieldrin, heptachlor epoxide, and the nonpesticide polychlorinated biphenyl (PCB) (*2*).

In a Swedish study of human milk (*28*), the levels of polychlorinated dioxins (PCDs) and polychlorinated dibenzofurans (PCDFs) were found to have declined

Table 1. Examples of biomarkers of exposure to some pesticides.

Indicator	Pesticide	Reference
Urinary residues of pesticides and their metabolites	Chlordane and heptachlor	Curley and Garrettson (*56*)
	DDT	Morgan and Roan (*57*)
	Deltamethrin	He et al. (*58*)
	Dichlorvos	Das et al. (*59*)
	Permethrin	Rhee et al. (*60*)
	Pentachlorophenol	WHO/Ahlberg et al. (*61,62*)
	Atrazine	Catenarri et al. (*63*)
Adipose tissue residues	Chlordane and heptachlor	Sorocooland Lewis (*64*)
	DDT	Roan (*65*)
Blood residues	Aldicarb	Lee and Ransdell (*66*)
	Chlordane and heptachlor	Saito et al. (*67*)
	DDT	Morgan and Roan (*57*); Kriss et al. (*68*)
	Dichlorvos	Blair et al. (*69*)
	Pentachlorophenol	WHO (*61*)
Breast milk residues	Organochlorine pesticides	Basri-Ustunbas et al. (*70*)
	Chlordane and heptachlor	WHO (*71*); WHO (*72*)
	Organochlorine pesticides	Johansen et al. (*73*); Larsen et al. (*74*); Furst et al. (*75*)
Skin residues	Aldicarb	Lee and Ransdell (*66*)
Cholinesterase determinations	Aldicarb	Mortensen (*76*); Burgess et al. (*77*)
	Dichlorvos	Slomka and Hine (*78*)
	Fumigants	Garry et al. (*79*)
Increased blood coagulation time	Anticoagulant rodenticides	Walker (*29*)

during the previous 10 years. It was suggested that the decline was partly due to the prohibition in Sweden of the use of certain pesticides such as chlorinated phenols. These pesticides contain various amounts of toxic PCDs and PCDFs as contaminants.

Biochemical responses to environmental chemicals (biochemical biomarkers) provide a measure of toxic effect. They are particularly valuable when used to measure the toxic effects of chemicals in the field, employing nondestructive sampling methods. A widely used biochemical biomarker is cholinesterase depression, which may involve destructive sampling (brain acetylcholinesterase [AChE]) or nondestructive sampling (serum butyrylcholinesterase). With a specific antibody it is possible to measure the concentration of a particular esterase in plasma or serum. Diagnostic kits to measure specific activities of blood esterases would be of considerable value in measuring exposure to organophosphorus insecticides in the field (*29*).

Measurement of AChE in red blood cells (RBCs) is widely used to assess cholinergic effects, whereas inhibition of neuropathy target esterase in lymphocytes might be used to assess the delayed neurotoxic effects of some organophosphate insecticides (*30–32*).

A semiquantitative tintometric field kit has been used in the developing world for almost 30 years to measure whole blood cholinesterase levels in persons exposed to organophosphate pesticides. The validity of this screening kit was evaluated among 79 workers heavily exposed to organophosphates by comparison with a reference assay for erythrocyte cholinesterase. Overall correlation between the two methods was good. However, either sensitivity or specificity of the tintometric kit was less than 75% for each of the three tintometric categories commonly used to define the limit of normal (*33*).

A clinic-based study of erythrocyte cholinesterase levels, pesticide exposures, and health effects was conducted among farm workers and non-farm workers to determine risks for exposure and associated morbidity. A total of 202 farm workers and 42 non-farm workers were recruited sequentially at two community health centers. Erythrocyte cholinesterase levels were measured colorimetrically. Farm workers had cholinesterase levels significantly lower than those of non-farm workers, although only sprayed pesticides were associated with very low levels (*34*).

Panemangalore and Byers (*35*) tried to quantitate dermal and respiratory exposure of field workers while applying maleic hydrazide (MH-30) and acephate (AP) to tobacco and reentering fields. AP, MH-30 and metabolites, cadmium (Cd), and plasma AChE in blood, plasma, and urine of farm workers (pre- and postexposure) were analyzed to assess the risk. Aminolevulinic acid dehydratase (δ-ALAD) activity and enhancement of calcium permeability in human erythrocytes as putative biomarkers of exposure of Cd, AP, and MH-30 were evaluated. Plasma cholinesterase, erythrocyte superoxide dismutase (SOD), and δ-ALAD activities were inhibited by 32, 56, and 32%, respectively, after 30 days of working on sprayed fields; RBC Zn decreased by 35%. From the enzyme inhibition data it is apparent that the field workers were exposed to significant levels of synthetic and natural toxicants. The relationship between RBC, SOD, and Zn suggests a compromise of the antioxidant enzyme status.

The detection of biochemical changes caused by anticoagulant rodenticides provides another example of this approach (e.g., monitoring of changes in vitamin K cycle or monitoring increases in precursors of clotting proteins in blood) (*29*).

Analysis of blood serum amino acid metabolism parameters may be used as diagnostic criteria of metabolic disorders in a body exposed to pesticides. Amino acid metabolism was studied in subjects occupationally exposed to pesticides for a long time. Reduced levels of taurine, cystine, methionine, and alanine and increased levels of phenylalanine were revealed in subjects after prolonged exposure to pesticides (*36*).

For genotoxic chemicals, techniques that measure DNA damage (e.g., detection of DNA adducts) provide a powerful tool in measuring environmental effects (*29,37*). Adducts to hemoglobin have been detected with several pesticides (*38*). The advantages of such measurements include the possibility of assessing dose closer to the target, of assessing individual capacity to form electrophils, and of extrapolating data on toxicity more easily across species. When the mechanism of actions of a pesticide is understood, more specific markers can be used (*5*).

In cord blood, metabolites of pesticides can be detected (*39*). New biomarkers may provide important information on the transplacental transfer of genotoxic compounds (*6*).

Determination of chromosomal aberrations (CAs) in cultured lymphocytes is an established method of monitoring populations occupationally or environmentally exposed to known or suspected mutagenic–carcinogenic agents (*40*). Cytogenetically visible damage in human chromosomes can be detected as sister chromatid exchanges (SCEs) or as micronucleated cells (*41*).

Several studies are described on the cytogenetic effects of workers in several countries who handled various pesticide formulations. Only in the case of ethylene dibromide and phosphine was exposure to a single, identified insecticide observed. No cytogenetic effect was observed with exposure to ethylene dibromide (*42,43*), while a significant excess of CAs was observed in most other studies of workers handling not only a mixture of insecticide formulations but also other pesticide formulations. The majority of these studies reported increases in the frequency of CAs or SCEs among the exposed workers. The frequency of chromatid breaks in samples taken from 16 agriculture workers (crop dusters, formulators, spray ring operators, farmers) in the United States was higher (1.56 ± 0.29) than in the 16 controls (0.44 ± 0.22). Workers were exposed to organophosphates, organochlorines, phenolics, and carbamates (*44*).Thirty-six floriculturists in Argentina involved in spraying various insecticides including organophosphates, organochlorines, and carbamates were studied. A significant increase in the frequency of CAs and SCEs was observed among the sprayers compared to the control group (*45*). In Hungary, 80 workers involved in mixing and spraying pesticides (80 formulations: insecticides, herbicides, and fungicides) were studied. A significant increase in the frequency of CAs was seen in the exposed group as compared with controls (*46*).

Chromatid breaks were significantly increased in vineyard workers from India. They were exposed to various pesticides used throughout the year including seven insecticides (DDT, lindane, quinalphos, metasystox, parathion, dichlorvos, and dieldrin) and two fungicides (*7*).

Fifty-five farmers and greenhouse workers from Hungary were studied. They were exposed to various formulations of agrochemicals (insecticides: organophosphates, carbamates, pyrethroids; and fungicides). A slight increase in the frequency of CAs was recorded compared with the controls (*47*).

Workers who mixed and sprayed pesticides in cotton fields in India were studied. They were exposed to insecticides including DDT, malathion, fenvalerate, cypermethrin. The frequency of CAs (*48,49*) and SCEs (*50*) was significantly increased in the

exposed groups. There was a positive trend with duration of exposure.

Fumigant applicators of phosphine in the grain industry had a 5-fold higher frequency of chromosomal deletions than control subjects. The frequency of breaks was also significantly increased. Chromosomal banding analysis showed that chromosomal rearrangements were 6 times more frequent than in the controls (*51*).

In another study, workers who apply pesticides and are exposed to the fumigant phosphine, or who have a mixed exposure to other pesticides and phosphine, demonstrate a significant increase in chromosome rearrangements in G-banded chromosomes from peripheral blood compared to control subjects. Workers who had discontinued using phosphine for at least 8 months prior to specimen collection do not demonstrate significant increases in chromosome rearrangements compared to controls. Breakpoint analysis demonstrates 5.4% breaks in exposed subjects compared to 4.0% in control subjects. Bands with significantly more breaks than expected in all study subjects were 1q32, 3p14, 7p15, and 14q11. Three of these four bands had significantly more breaks than expected in the exposed group, and all four bands had a significant excess of breaks in the control group. There are four bands with a significant excess of breaks in the exposed group and no breaks in the control group; each of these occurs in a known protooncogene region. These are 1p13 (*NRAS*), 2p23 (*NMYC*), 14q32 (*ELK2*), and 21q12 (*ETS-2*). Most breaks at bands 1p13, 14q32, and 21q22 are associated with chromosome rearrangements and occurred in appliers who have a mixed exposure to phosphine and other pesticides. Cytogenetic abnormalities, i.e., rearrangements or deletions involving bands 1p13, 2p23, and 14q32, are associated with non-Hodgkin's lymphoma. These findings could relate to the risk of evolution of a neoplastic clone in these workers. Epidemiologic studies of similarly exposed workers indicate an excess of non-Hodgkin's lymphoma (*52*).

The effect of exposure to the chlorinated cyclodiene termiticide aldrin was evaluated in pest control workers potentially exposed to this material. Sister chromatid exchange (SCE) frequencies were not elevated in workers handling aldrin. This is consistent with the fact that chlorinated cyclodienes are not genotoxic. Plasma dieldrin concentrations (up to 250 ng/ml) confirmed exposure in workers actively performing termiticide treatments and in maintenance and store workers compared with unexposed control workers (median concentration, 4.8 ng/ml). Urinary D-glucaric acid (DGA), an index of hepatic enzyme activity, was elevated in pesticide-exposed groups but urinary DGA was poorly correlated with plasma dieldrin level. This indicates that concurrent exposures of these groups to other pesticides may have influenced mixed-function oxidase metabolic activity (*53*).

Discussion

It is critical for the design, implementation, and evaluation of such studies that epidemiologists and biostatisticians be familiar with methodological issues relevant to the direct measure of exposure. Quality control and assurance, statistical sampling, and the limitations of these studies should be considered (*54*).

The process of selection and validation of biomarkers for monitoring and screening requires careful consideration of the relevance and accuracy of the tests. The extent to which individuals are at higher risk depends on the strength of the association between a positive result and the risk of developing the adverse health effects. The result of the test is related not only to exposure level and biological and environmental variability but also to the accuracy of the test that depends on its reliability and validity. Reliability depends on the standardization of techniques and the quality control of procedures. Validity is the extent to which the test measures what it is intended to measure, or its sensitivity and specificity (*20*).

During risk assessment of exposure to pesticides, we should consider the following important issues:

a) Biomarkers are predictive assays rather than diagnostic. A positive effect will be indicative of exposure, but cannot be considered predictive of the future occurrence of any particular change in phenotype such as cancer.

b) The criteria for determining a positive result should consider statistically significant dose-related increase in the biomarker. There is lack of a satisfactory theory of dose–response relationships to allow extrapolation quantitatively from data obtained at rather high dose level.

c) Humans are not exposed uniformly to single, pure chemicals, but rather to complex and variable mixtures of substances; some of these substances have antimutagenic activity while others may interact synergistically. In most human exposure situations, a single xenobiotic chemical dominates the environment but others are always present, even in low concentrations.

d) Humans possess a number of metabolic processes that may eliminate, detoxify, or possibly activate certain chemicals. There are various DNA repair mechanisms that remove potentially mutagenic lesions from DNA. The efficiencies of these repair processes vary from lesion to lesion, tissue to tissue, and person to person. Thus, human populations are heterogeneous with respect to mutagen sensitivity.

Finding answers to many of the key challenges, will continue to be a major factor in investigating the role, if any, of these chemicals in the transmission of human diseases. These challenges inslude accurate exposure assessment, pesticides' role in multifactorial causation of disease, latency, publishing of negative results, misclassification, low-level dose measurement, accurate diagnosis, the role of biomarkers, adequate study design, and adequate funding.

Expanding the repertoire of available biomarkers of pesticide exposure and employing multiple ones in well-designed study protocols will provide critical tools in the evaluation of pesticide safety and design of appropriate measures to minimize adverse exposures. Therefore, the combination of *in vitro*, animal, and human data will give the best picture of a marker's performance (*55*).

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